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Direct soil organic matter compound specific $\delta^{13}\text{C}$ analysis using pyrolysis (Py-CSIA): identification of biomarkers in a *dehesa* from Southern Spain

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Highlights:

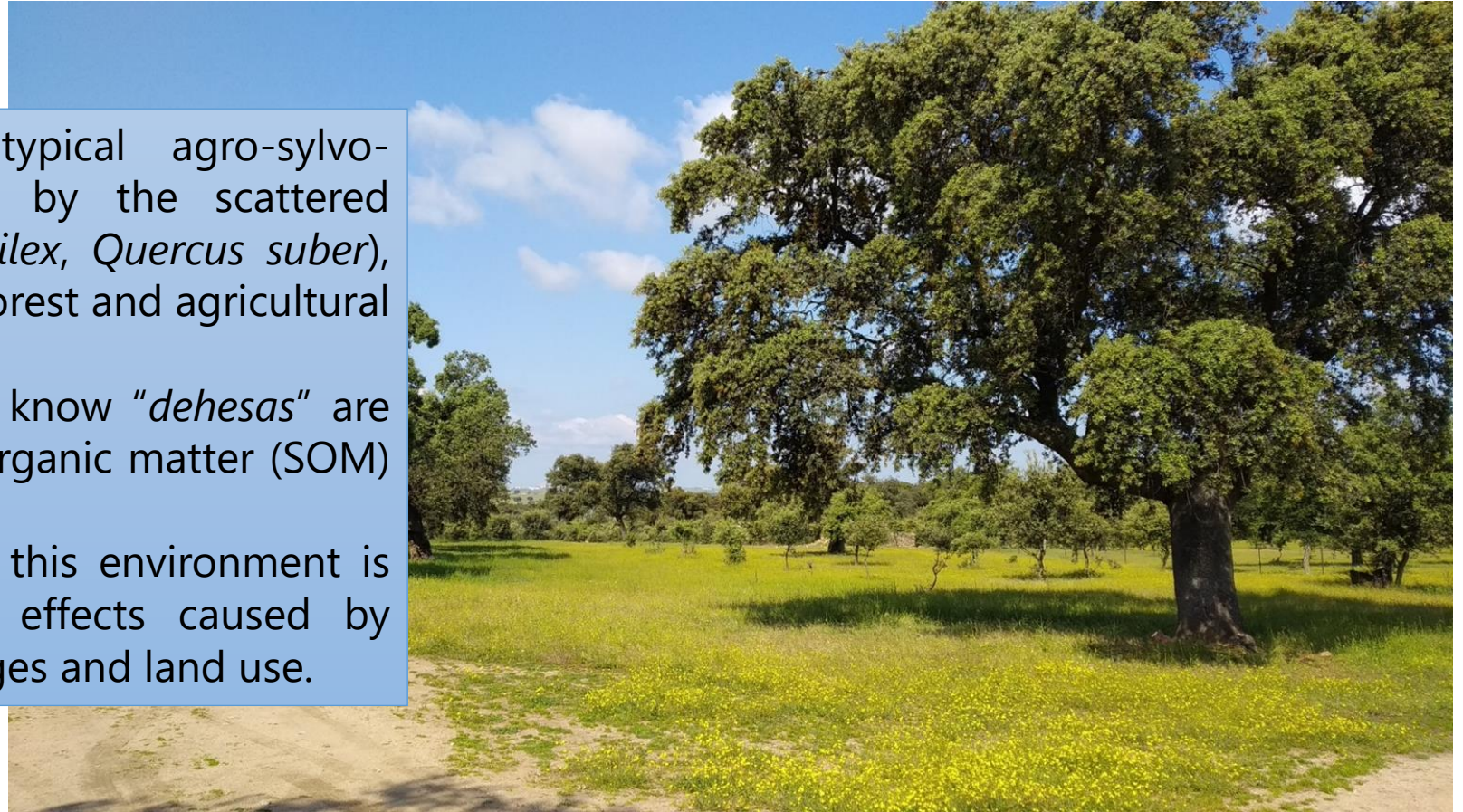
In this display we show the possibility of using this particular analytical pyrolysis technique (Py-CSIA) for the direct measurement of $\delta^{13}\text{C}$ in relevant specific soil organic matter components including those from polysaccharides (cellulose/hemicellulose), lignin, lipid/waxes and also peptide/protein derived compounds.

According to the content showed in the following slides, we can conclude this approach can help to monitor SOM turnover rates according to the progressive transformation of compound families.

Soil organic matter in *dehesas*



- Mediterranean savannas are typical agro-sylvo-pastoral systems, characterized by the scattered presence of oak trees (*Quercus ilex*, *Quercus suber*), and the integration of livestock, forest and agricultural practices.
- The particularity of these locally known "*dehesas*" are expected to be reflected in soil organic matter (SOM) structure.
- Understanding SOM dynamic in this environment is important to forecast possible effects caused by climatic, and environmental changes and land use.



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How did we proceed?

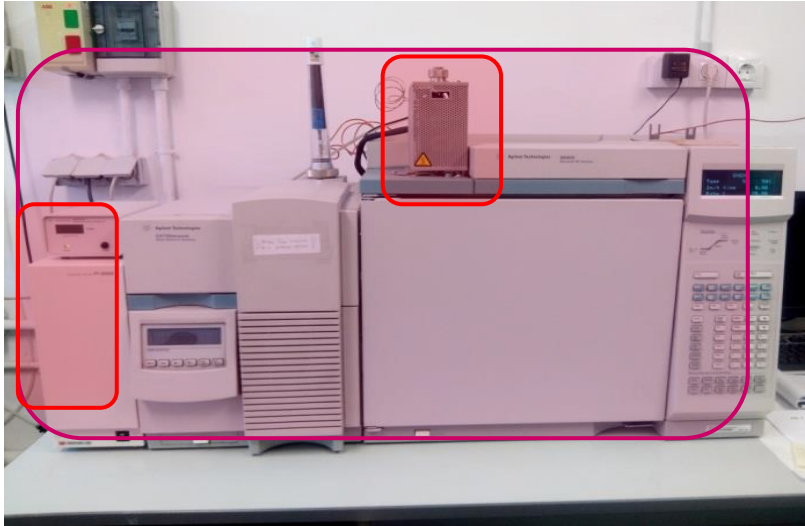


In April 2019, soil samples were taken from the 10 uppermost cm of soil in an experimental plot (under evergreen oak canopy), in collaboration with the project DECAFUN (CGL2015-70123-R). Sampling was conducted in Pozoblanco (Córdoba, Spain).

- We evaluated molecular composition of soil organic matter (SOM) from bulk soil samples, using online analytical pyrolysis coupled to gas chromatography-mass spectrometry analysis (Py-GC/MS) at 400°C.
- Furthermore, we aimed to estimate molecular turnover times of major SOM components (lignin-derived units, carbohydrates, phenols, fatty acids, etc.) using compound-specific isotope analysis (Py-CSIA), under the same chromatographic conditions.

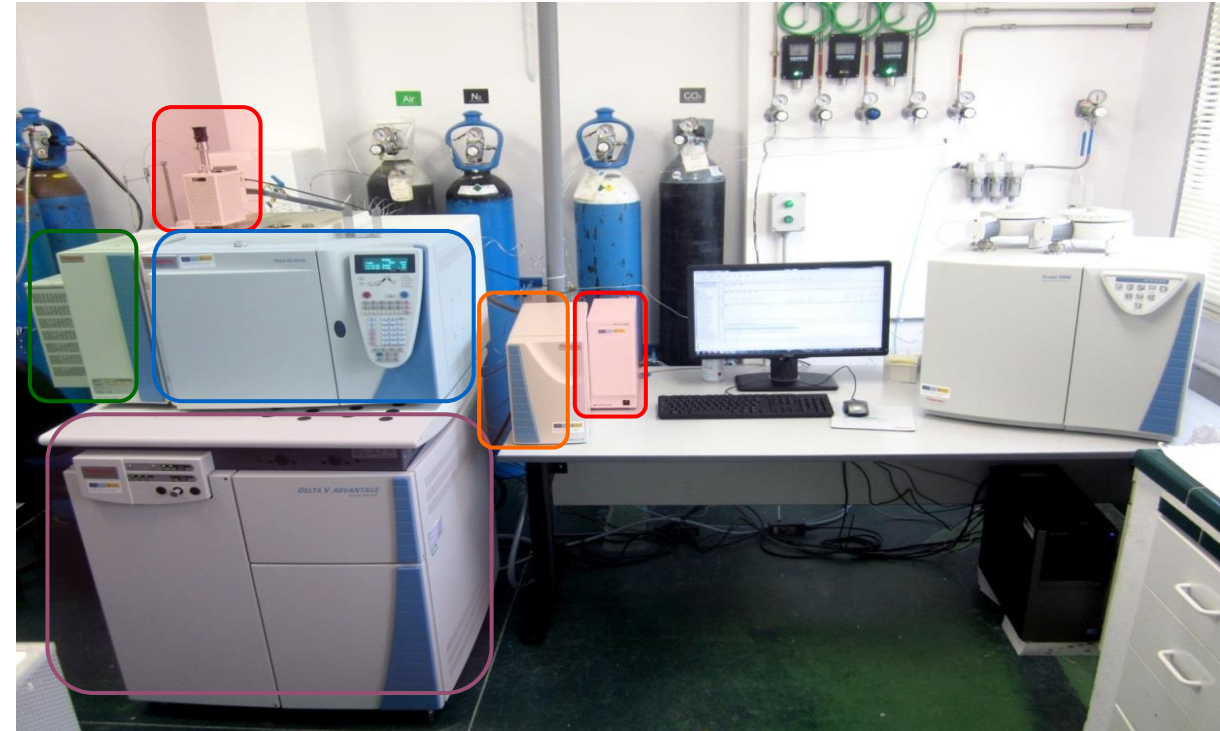
Possibilities of direct pyrolysis CSIA in bulk soil samples?

Lab equipment



Py-GC/MS: structural information

A conventional Py-GC/MS system (pyrolyzer F. Labs 2020i) + Agilent MSD 6890N



Py-GC-C/TC-IRMS: isotopic information ($\delta^{13}\text{C}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}$)

A double-shot/micro-furnace pyrolyzer (Frontier Laboratories, model 3030iD)

Gas chromatographer (Trace Ultra GC system)

Individual volatile pyrolysis products separated by GC are directed to GC-Isolink System with combustion (C and N) and pyrolysis (H or O) micro-furnaces.

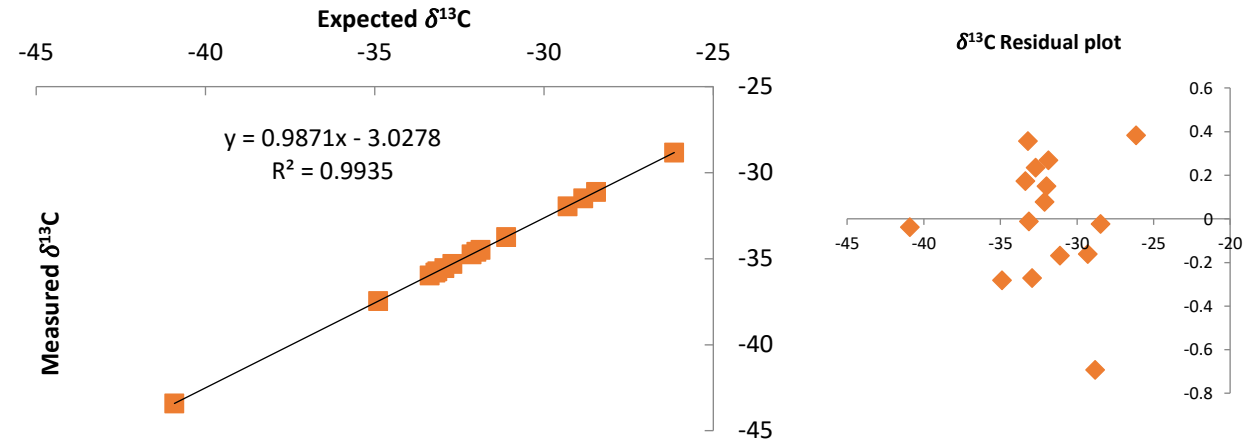
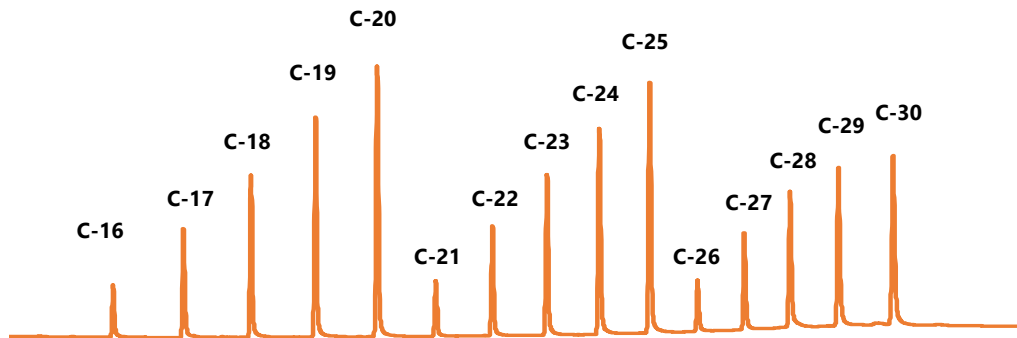
Coupled to isotope ratio mass spectrometer, IRMS (Thermo Delta V Advantage) via a ConFlo IV universal interface unit.



Testing Py-CSIA method



$\delta^{13}\text{C}$: 5 μL (dil. 1:25) 400°C – B5 n-alkane mixture



The system was externally calibrated prior to any analysis, using standard mixtures C4 for n-alkanes (n-C₁₆ to n-C₃₀), obtained from the Stable Isotope Laboratory (Schimmelmann, Indiana University) <http://arndt.schimmelmann.us/compounds.html>

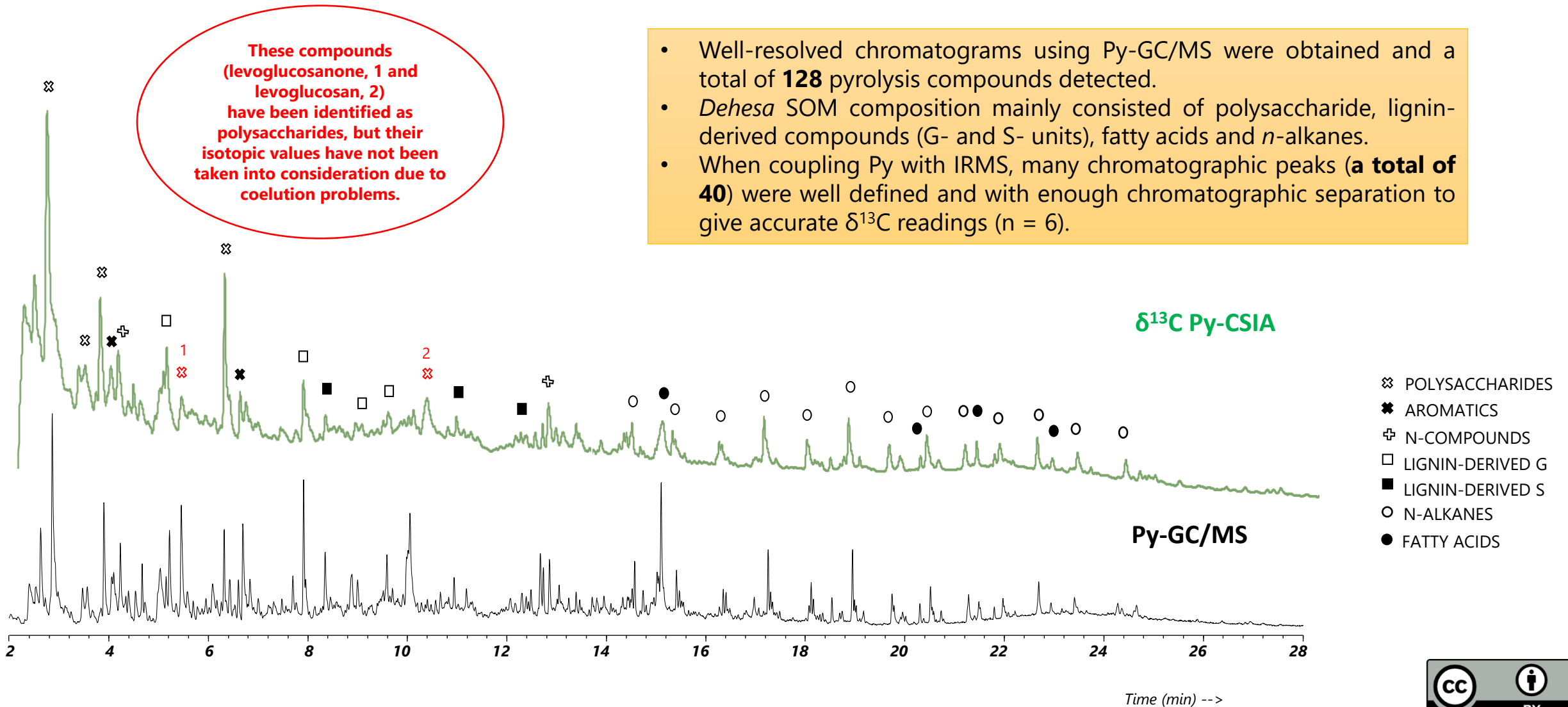
The values obtained were accepted when fitting accordingly to a straight line ($R^2 > 0.99$).

Chromatographic results

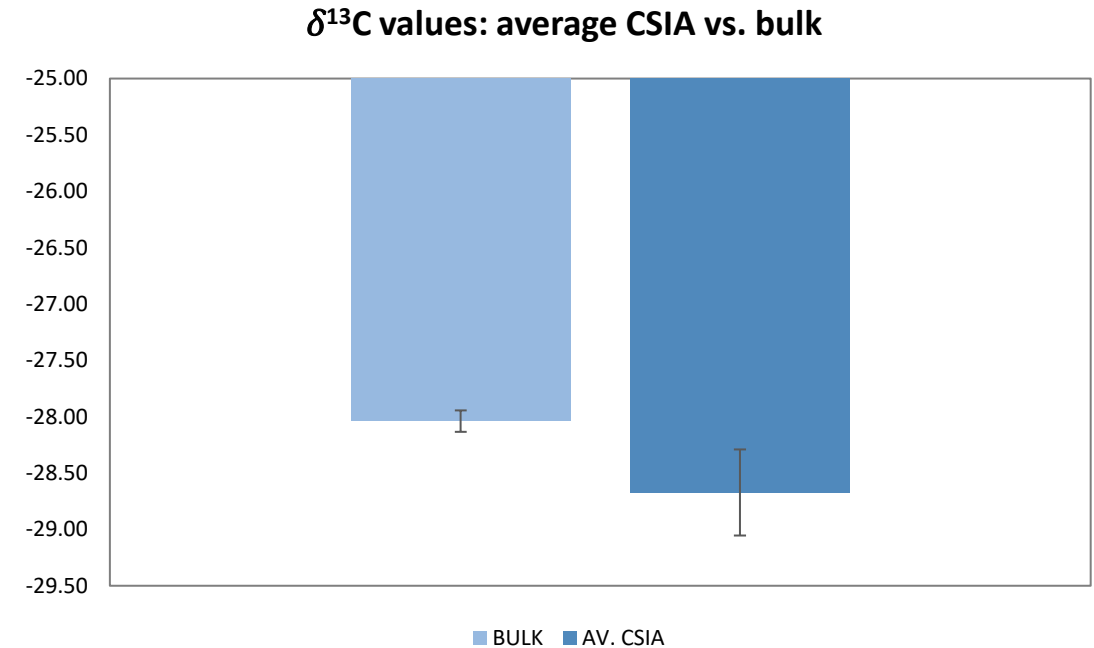
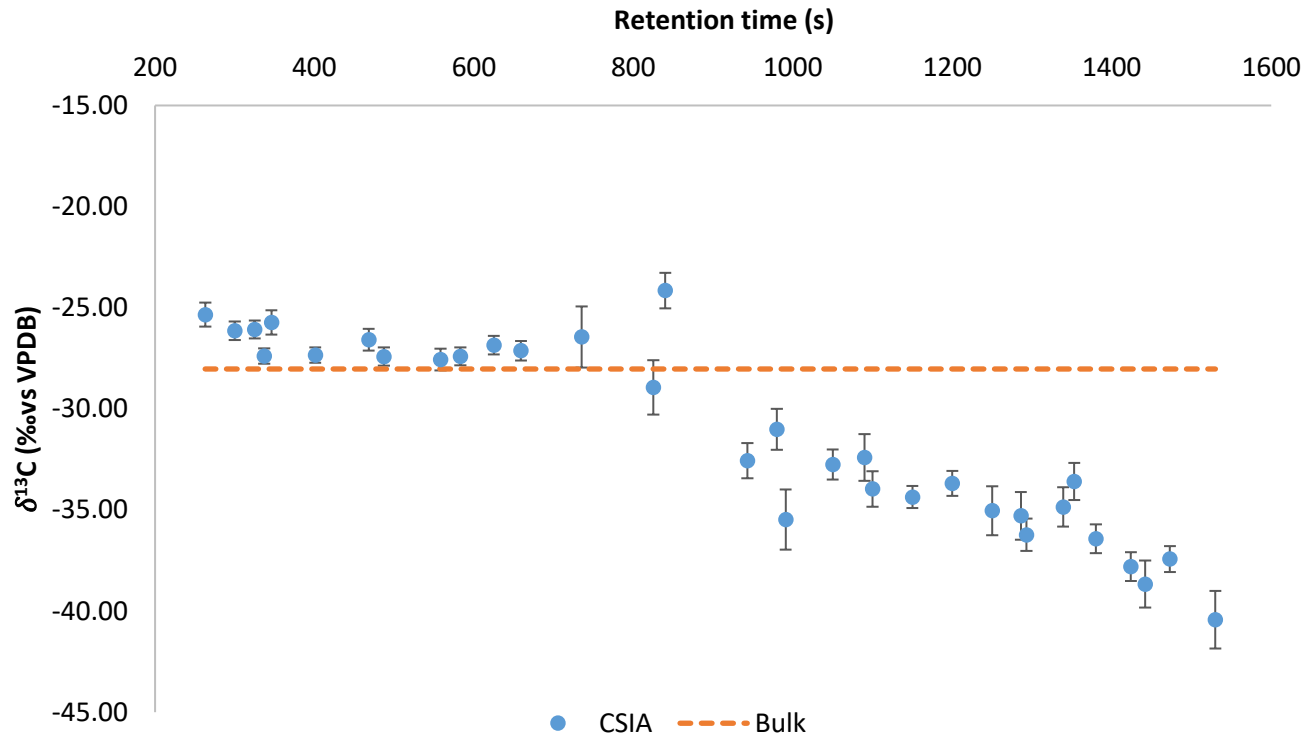


These compounds
(levoglucosanone, 1 and
levoglucosan, 2)
have been identified as
polysaccharides, but their
isotopic values have not been
taken into consideration due to
coelution problems.

- Well-resolved chromatograms using Py-GC/MS were obtained and a total of **128** pyrolysis compounds detected.
- *Dehesa* SOM composition mainly consisted of polysaccharide, lignin-derived compounds (G- and S- units), fatty acids and *n*-alkanes.
- When coupling Py with IRMS, many chromatographic peaks (a **total of 40**) were well defined and with enough chromatographic separation to give accurate $\delta^{13}\text{C}$ readings ($n = 6$).



$\delta^{13}\text{C}$ CSIA and bulk values



- Py-CSIA show isotopic shifts along the running sequence of the soil sample, corresponding to the diverse compound families.
- Average CSIA isotopic values agrees consistently with the bulk isotopic value, where no significant statistical differences were observed.
- Standard deviation of CSIA value is higher, mainly due to intramolecular isotope distributions and potential coelution problems in terms of chromatographic resolution.

$\delta^{13}\text{C}$ CSIA according to SOM compounds

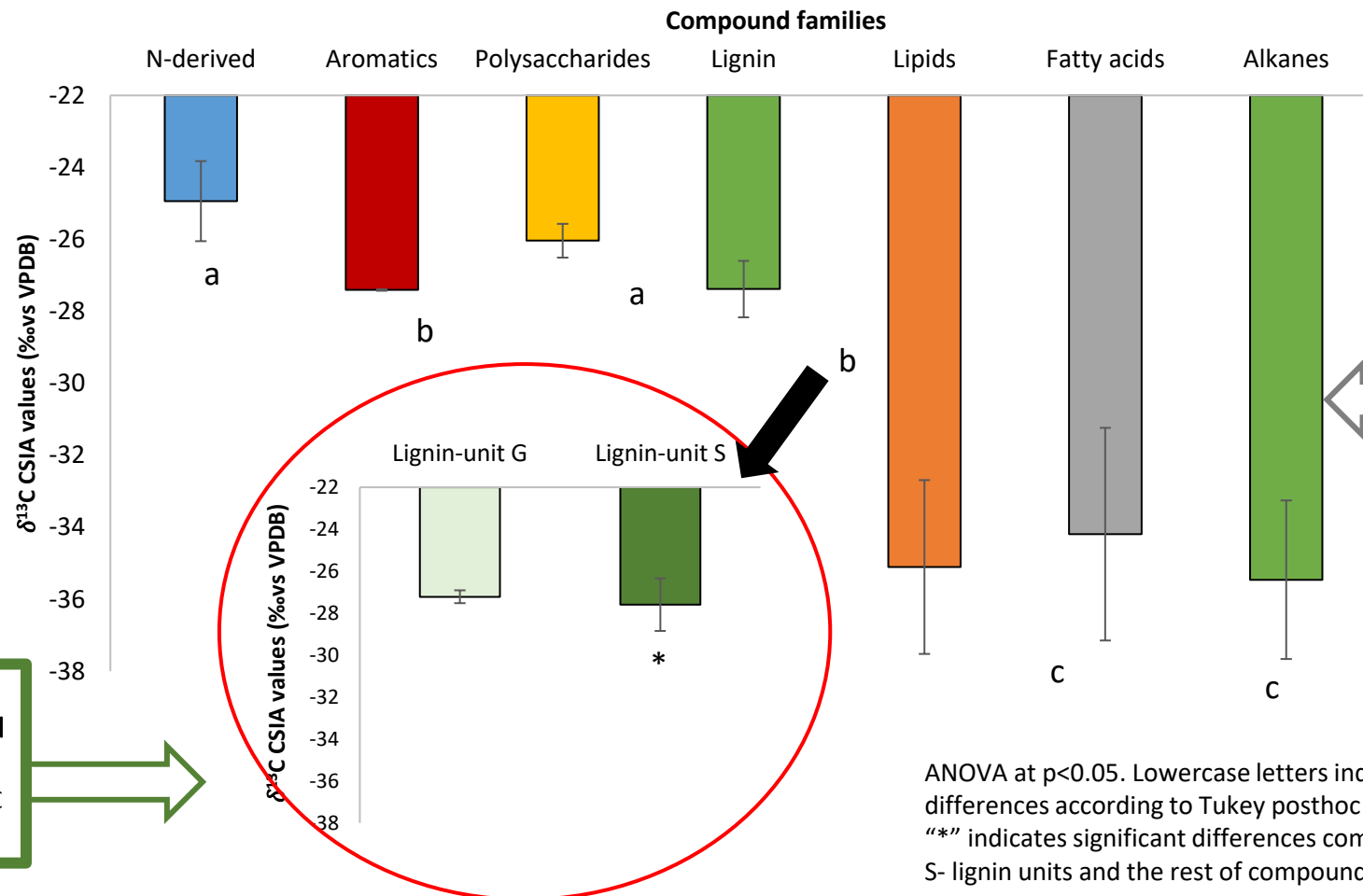


Polysaccharide derived pyrolysis products (furfural, cyclopentanedione), showed slightly enriched $\delta^{13}\text{C}$ values ($-26.0 \pm 0.47 \text{ ‰}$) that is according to their naturally ^{13}C enriched chemical structure [2].

The $\delta^{13}\text{C}$ values recorded for aromatic compounds (catechol, phenol) ($-27.41 \pm 0.02 \text{ ‰}$) provides information about their natural origin as pyrolysis-derived precursors of methoxyphenols [3].

Statistical differences were also observed in lignin-derived units (methoxyphenols), showing slightly depleted $\delta^{13}\text{C}$ values ($-27.4 \pm 0.78 \text{ ‰}$).

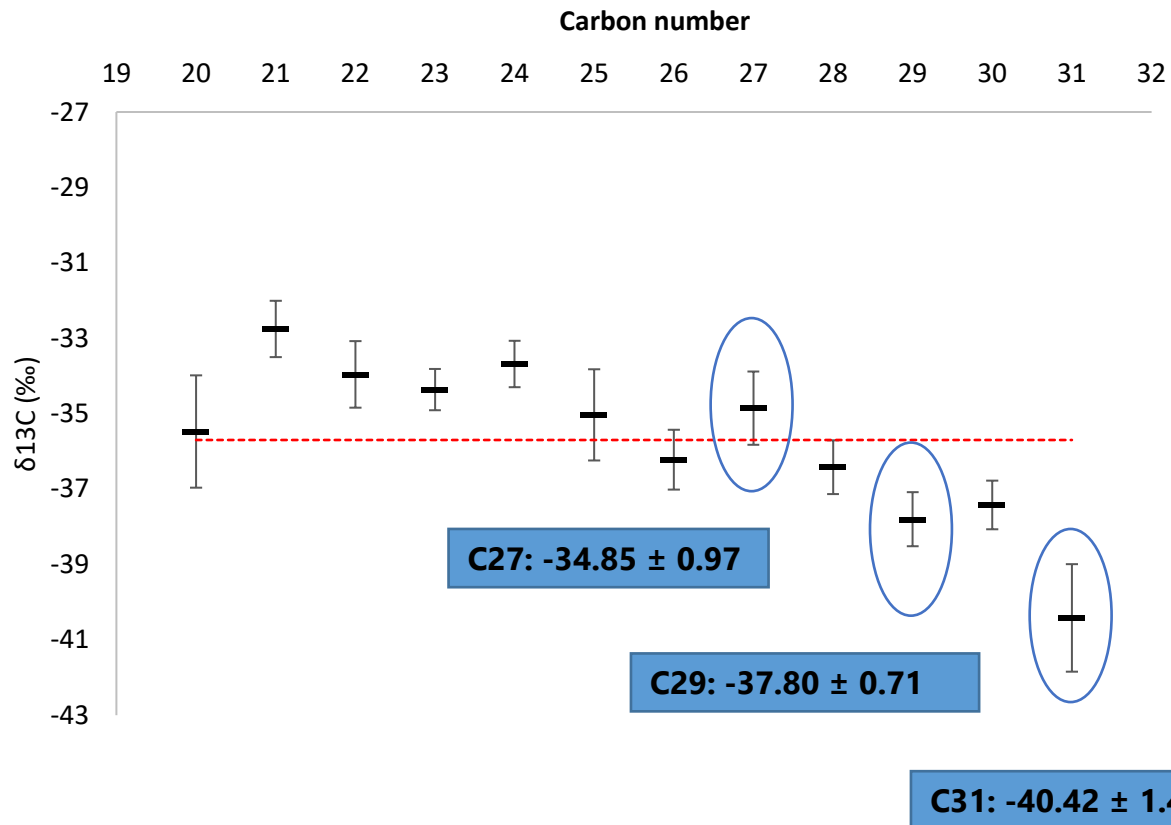
The carbon isotope composition for specific biomass compounds released after the pyrolysis of soil was found in line with the expected values for C3 plants i.e. *Quercus* spp [1]



Accordingly, depleted $\delta^{13}\text{C}$ values for fatty acids ($-35.1 \pm 2.41 \text{ ‰}$) and alkanes ($-35.5 \pm 2.20 \text{ ‰}$) were found, the latter with lighter isotope composition with increasing the hydrocarbon length.

ANOVA at $p < 0.05$. Lowercase letters indicate significant differences according to Tukey posthoc analysis. "*" indicates significant differences compared between S- lignin units and the rest of compound families.

n-Alkanes Py-CSIA distribution



- Average $\delta^{13}\text{C}$ isotopic composition of *n*-alkanes (-35.70 ± 2.25) fall into the range of C3 (higher) plants (carbon fixation pathway) [4].
- The carbon number distribution from the *n*-alkanes shows a range of C20 to C31, with larger relative abundance of odd chain compounds.
- Compound-specific isotopic composition of *n*-alkanes varied widely, from -32.2 to -39.9 ‰ accordingly to the carbon chain length, specially observed in the main odd *n*-alkanes used as biomarkers in SOM: **C27, C29 and C31**.

Conclusion remarks



- Compound-specific isotope analysis results in a useful technique for SOM source attribution, as SOM turnover estimation should not rely on bulk isotope values.
- Our results support the suitability of Py-CSIA for the direct measurement of $\delta^{13}\text{C}$ in relevant soil organic matter specific components, including those from polysaccharides (cellulose/hemicellulose), lignin, lipid/waxes and peptide/protein derived compounds.
- In chromatographic terms, special attention should be driven to potential co-elution problems in IRMS, especially when analytical pyrolysis is being used in complex samples.
- Our methodology described here has the potential for routine analysis and screening studies of soil samples to be complemented with conventional structural information in samples with high macromolecular complexity.
- Therefore, this approach can help to monitor SOM turnover rates according to the progressive transformation of compound families.

Thanks for your “virtual” attention!

Would you like to know more?

Here you have some useful references:

- [1] Simón, N., Montes, F., Díaz-Pinés, E., Benavides, R., Roig, S., & Rubio, A. (2013). Spatial distribution of the soil organic carbon pool in a Holm oak dehesa in Spain. *Plant and soil*, 366(1-2), 537-549.
- [2] González-Pérez, J. A., Jiménez-Morillo, N. T., de la Rosa, J. M., Almendros, G., & González-Vila, F. J. (2016). Compound-specific stable carbon isotopic signature of carbohydrate pyrolysis products from C3 and C4 plants. *Journal of the Science of Food and Agriculture*, 96(3), 948-953.
- [3] Poole, I., & Van Bergen, P. F. (2006). Physiognomic and chemical characters in wood as palaeoclimate proxies. In *Plants and Climate Change* (pp. 175-196). Springer, Dordrecht.
- [4] Wiesenberg, G. L., Hirave, P., Birkholz, A., & Alewell, C. (2019, January). Understanding the effects of early degradation on isotopic tracers: implications for sediment source attribution using compound-specific isotope analysis (CSIA). In *Geophysical Research Abstracts* (Vol. 21).

More info about our project [INTERCARBON](#)

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